## Remarks

The present invention is based on the discovery that recombinase-encoding nucleic acid constructs can be incorporated into the genome of embryonic stem (ES) cells to be later turned on and expressed in the development of transgenic organisms. These constructs are expressed at high levels in the germline of the transgenic organism by means of a germline specific promoter, without being expressed to a functionally significant extent in either ES cells (i.e., in culture) or in embryonic or adult somatic tissues.

Accordingly, the present invention provides transgenic mammalian embryonic stem cells whose genomes are transformed with recombinase-encoding nucleic acid constructs (claims 12-15, 18-24, 26 and 49-51) useful for preparation of transgenic animals, such as mice. These nucleic acid constructs comprise a mammalian germline promoter operatively linked to a recombinase encoding gene. The genome of such ES cells can further contain a transcriptionally active selectable marker flanked by recombinase recombination sites and/or a nucleic acid fragment flanked by recombinase recombination sites specific for a different recombinase than the recombination sites that flank the selectable marker. Because the germline-specific promoter directs recombination events in the germline, but only to a *de minimis* amount in other tissues, embryos can be derived from such ES cells that contain a transgenic allele, such as is caused by recombination at recombinase target site(s).

In addition, when the ES cells contain a nucleic acid fragment flanked by recombinase recombination sites specific for a different recombinase than the recombinase expressed in the germline (i.e., different than the recombination sites that flank the selectable marker), such as under control of an inducible or tissue specific promoter, recombination of the target site can be controlled to occur in a specific (e.g. somatic) tissue or in an inducible manner. In addition, because the ES cells contain a germline specific promoter operatively linked to the recombinase encoding gene, the transcriptionally active selectable marker can be excised by passage of the genome derived from said embryonic stem cells through gametogenesis (claims 28-31). ES cells obtained by crossing the genome of the transgenic gamete with a wild type genome can be used to

obtain ES cells in which the transgene is stably incorporated, into the genome, but the selectable marker is excised. Excision of the marker without excision of the allele of interest allows any phenotype that is observed to be more confidently ascribed to the mutation of interest rather than to some combination of that mutation and the transcriptionally active marker.

In accordance with the invention there are also provided methods for the production of transgenic animals (i.e., containing recombinant alleles; claims 32, 34-42, 44, and 46-48), as well as methods for conditional assembly of functional genes for expression in eukaryotic cells by recombination of individual inactive gene segments from one or more genes of interest using the invention ES cells containing recombinase responsive nucleic acids (claim 43). The methods described herein represent a novel method of general DNA manipulation with broad applicability to any gene of interest.

Claims 12-15, 18-24, 26, 28-32, 34-44, and 46-51 were pending before this communication. By this response, claims 12, 24, 26, 28, 32, 35, 40, 41, 43, 44 and 49-51 are amended. Attached hereto is a marked-up version of the changes made to the claims by the current amendment, labeled APPENDIX A. A clean copy of the complete set of pending claims for this application is also provided for the Examiner's convenience in APPENDIX B. These amendments add no new matter as all claim language is fully supported by the specification and the original claims.

The objection to claim 40 because of alleged informalities (specifically, allegedly confusing terminology) is respectfully traversed. Applicants submit that the claim language is clear to one of skill in the art. However, in order to reduce the issues and expedite prosecution of this application, claim 40 has been amended to place "first" and "second" in sequence (lines 7-8). Accordingly, Applicants respectfully request withdrawal of the objection to claim 40.

The rejection of claims 12-15, 18-24, 26 and 49-51 under 35 U.S.C. § 101 as allegedly being directed to non-statutory subject matter is respectfully traversed. Applicants disagree with

the Examiner's assertion that human embryonic stem cells are the equivalent of a human being or human embryos, and therefore non-statutory subject matter.

Contrary to the Examiner's assertion, in guidelines issued by the National Institute of Health, human embryonic stem cells are defined as human pluripotent stem cells, in turn defined as "cells that are self-replicating, are derived from human embryos or human fetal tissue, and are known to develop into cells and tissues of the three primary germ layers. Although human pluripotent stem cells may be derived from embryos or fetal tissue, such stem cells are not themselves embryos." (National Institutes of Health Guidelines for Research Using Human Pluripotent Stem Cells, 65 Fed. Reg. 51,976 (2000), emphasis added).

However, to reduce the issues and expedite prosecution of this application, and not for reasons of patentability, claims 12, 26, 49 and 51 have been amended to encompass only non-human mammalian embryonic stem cells, which the Examiner acknowledges overcomes this rejection (Paper No. 21 at page 3, paragraph 2.). Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejections of claims 12-15, 18-24, 26 and 49-51 under 35 U.S.C. § 101.

The rejection of claims 32, 34-43, and 46-48 under 35 U.S.C. § 112, first paragraph, as allegedly lacking adequate written description is respectfully traversed. Applicants respectfully disagree with the Examiner's assertion that the terms "gene segments" and "nucleic acid fragment" require a description of the DNA itself to provide an adequate written description (Paper No. 21 at page 3, paragraph 3.). Indeed, it is respectfully submitted that the described methods do not require specific DNA sequences to be identified. In one aspect of the present invention, Applicants have overcome a need in the art for tissue-specific and conditional recombinatory tools to create transgenic animals and plants. The methods claimed to practice this invention entail a general manipulation of DNA, meant to have broad applicability and are useful for the introduction of any gene of interest. Nucleic acid fragments contemplated for use with these methods include any fragments containing at least a portion of a gene of interest (see specification at page 13, lines 11-13).

As such, it is the methods themselves, and not any particular sequence of DNA that is claimed herein. An adequate description of the method then requires only that the fragment of DNA (i.e. the gene of interest to be manipulated) be identified as a nucleic acid fragment (claims 32, 34-42, and 46-48), or a gene segment (claim 43). In contrast to both *Fiers v. Revel*, 25 USPQ2d 1601 (CAFC 1993) and *Regents of the Univ. Calif. v. Eli Lilly & Co.*, 43 USPQ2d 1398 (CAFC 1997), the present invention is not directed to the isolation of any specific DNA sequence, but rather for the manipulation of a DNA fragment or segment already in hand. Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejection of claims 32, 34-43, and 46-48 under 35 U.S.C. § 112, first paragraph.

The rejection of claims 12-15, 18-24, 26, 28-32, 34-44 and 46-51 under 35 U.S.C. § 112, first paragraph, for alleged lack of enablement is respectfully traversed. Applicants respectfully submit that the claimed method is generally applicable to any mammalian cells, and is not restricted to use in mice. Indeed, the Examiner acknowledges that the specification is enabling for a mouse ES cell system utilizing the MP1 promoter operatively linked to a recombinase, wherein the resultant transgenic mouse expresses recombinase in its spermatid to a level that results in recombination in an embryo (Paper No. 21 at page 4, paragraph 4.).

The specification describes the preparation of pluripotent ES cells, introduction of exogenous nucleic acid constructs into these cells, and the transfer of these embryos into intermediate hosts or surrogate females for continuous development (see specification at page 9, lines 8-23). In addition, the specification identifies several germline-specific promoters contemplated for use in addition to the MP1 promoter (see specification at page 6, lines 1-12). The relationship among the members of the group is not that they are all mouse-derived promoters, but rather that they are all germline-specific for cells of sperm or oocyte lineage. If these promoters have been shown to be germline specific in the references cited, the Examiner provides no reason to doubt that they will retain such specificity when coupled to the gene being introduced, indeed having equivalent functionality to turn on gene expression in the target cells.

Furthermore, Applicants respectfully disagree with the Examiner's assertion that the MP1 promoter is not germline specific because it expresses protein in the heart, brain and spleen (Paper No. 21 at page 7, lines 2-3). Applicants respectfully submit that it is well known in the art that the term "tissue-specific" is indeed equivalent to the substantially exclusive expression as described in the specification (see specification at page 8, lines 15-18). Background levels of expression that are expected to be substantially lower (e.g., 100 fold lower at the highest) than observed in the specifically targeted cells, are not functionally significant (see, e.g., specification at page 23-25, Example 4).

Analogous to the range of promoters contemplated for use in the present invention, several recombinases are also intended, including several non-mammalian types of recombinases. These enzymes are known in the art to function well in mammalian assays of site-specific recombination, and their function under the influence of any of the above-mentioned germline-specific promoters would be easily determinable. For example, the inclusion of a marker gene as described in the present invention would easily monitor recombinase expression (see, e.g., specification at page 11, lines 6-21). Therefore, it would not require one of skill in the art to undertake undue experimentation to obtain any mammalian ES cell with any germline-specific promoter by monitoring recombinase expression in the target cells.

Applicants respectfully disagree with the Examiner's assertion that an ES cell comprising a nucleic acid sequence encoding recombinase operatively linked to a mammalian germline-specific promoter and further comprising yet another nucleic acid fragment or construct, as identified in claims 13-15, 24 26, 28-32, 34-43, 46-48, is not enabled (Paper No. 21 at page 7-8, bridging paragraph). It is well known in the art that the method of addition of such a second or third nucleic acid fragment or construct is identical to that described for the first nucleic acid fragment or construct as described in the specification (see, e.g., specification at page 9, lines 8-18). Therefore, any number of DNA constructs could be introduced into the ES cells simultaneously.

Applicants respectfully submit that the phenotype of such an animal could readily be monitored by either looking for expression of the introduced DNA (e.g. the marker gene, or a second type of recombinase) or by using PCR analysis to check for introduction of the introduced DNA into the genomic DNA. These methods are described in detail in the specification (see, e.g., Example 4 at pages 23-25). It is not necessary to predict the level of recombinase expression or the phenotype of the resulting animal before doing the actual manipulations of the ES cells, since they are readily determined following introduction of the DNA of interest. In addition, the resultant ES cells are clearly useful for generating novel transgenic animals containing an array of sophisticated mutations (see specification at page 15, lines 16-35). Therefore, ES cells containing multiple constructs or nucleic acid fragments as claimed in claims 13-15, 24 26, 28-32, 34-43, 46-48 are fully enabled by the teachings of the specification, especially in view of the detailed non-limiting examples provided.

Applicants respectfully disagree with the Examiner's further assertion that claim 43 is not enabled because the specification does not teach any inactive segments which can be used to make a eukaryotic cell of interest as claimed (Paper No. 21 at page 9-10, bridging paragraph). As discussed above, the method itself is being claimed, and the invention is in the broad applicability of such a method to any gene of interest. The inactive gene segment(s) becomes active in the presence of the recombinase which facilitates the site-specific recombination event (see specification at page 15, line 30 - page 16, line 4).

In view of the foregoing arguments, Applicants respectfully submit that all pending claims are fully enabled by the teachings of the specification. Accordingly, reconsideration and withdrawal of the rejection of claims 12-15, 18-24, 26, 28-32, 34-44 and 46-51 under 35 U.S.C. § 112, first paragraph, for lack of enablement are respectfully requested.

The rejection of claims 28-32, and 34-39 under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite is respectfully traversed. Applicants respectfully disagree with the Examiner's assertion that the phrase "passaging the genome derived from said embryonic stem cells through gametogenesis" is unclear (Paper No. 21 at page 10, paragraph 5.). Applicants

submit that the language is consistent with use in the field, and thus the terminology is clear to one of skill in the art. The specification further defines the phrase as spermatogenesis or oogenesis (see specification at page 11, lines 30-32).

In addition, by the present communication, claim 28 has been amended to clarify that the claimed method results in excision of the transcriptionally active selectable marker. Similarly, claim 35 has been amended to clarify that the claimed method results in the production of the recombinant allele.

In addition, claims 32 and 35 have been amended to clarify that the introduction of the ES cells of claim 12 or 26 is contemplated, and not the introducing step itself. Claim 43 has been amended to clarify that recombinase expression is required for conditional assembly to occur. Also, claims 49-51 have been amended to correct an obvious typographical error and correlate the preamble of these claims to that of claim 12, and direct the claims to ES cells and not a method. Typographical errors have also been corrected in claims 12, 26, 40, 41 and 44 to provide a consistent spelling of the word "germline".

In view of the foregoing amendments and remarks, Applicants respectfully submit that all pending claims are definite and particularly point out and distinctly claim the subject matter which Applicants regard as their invention. Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejection of claims 28-32, 34-44 and 46-51 under 35 U.S.C. § 112, second paragraph.

## Conclusion

Applicants respectfully request reconsideration of the rejections of the claims, and that a timely Notice of Allowance be issued in this case. In the event any matters remain to be resolved in view of this communication, the Examiner is encouraged to call the undersigned so that a prompt disposition of this application can be achieved.

Respectfully submitted,

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Enclosures

## APPENDIX A – ALTERED CLAIMS

## VERSION WITH MARKINGS TO SHOW CHANGES MADE

(Twice amended) **Non-human mammalian** **[~~Mammalian~~]** embryonic stem cells containing a nucleic acid construct comprising a mammalian germ**[-]**line-specific promoter operatively associated with a recombinase coding sequence, wherein the nucleic acid construct is in the genome of the stem cells and wherein the recombinase is not expressed in the stem cells in cell culture.

24. (Twice amended) Embryonic stem cells **[~~containing a nucleic acid construct~~]** according to claim 12 further comprising an inducible promoter operatively associated with a recombinase coding sequence and a transcriptionally active selectable marker flanked by two recombinase recombination target sites in the genome of the stem cells.

26. (Thrice Amended) **Non-human mammalian** **[~~Mammalian~~]** embryonic stem cells comprising a germ**[-]**line-specific promoter operatively associated with a recombinase coding sequence and a transcriptionally active selectable marker flanked by two recombinase recombination target sites in the genome of the stem cells.

28. (Thrice Amended) A method for excision of the transcriptionally active selectable marker from the embryonic stem cells of claim 26, said method comprising:

passaging the genome derived from said embryonic stem cells through gametogenesis**, wherein said passaging causes** **[~~to cause~~]** excision of the transcriptionally active selectable marker.

32. (Thrice Amended) A method for the production of recombinant alleles in a transgenic non-human animal, said method comprising:

introducing a nucleic acid fragment flanked by at least two recombinase recombination target sites into mammalian embryonic stem cells **of** **[~~according to~~]** claim 12; and

passaging the genome derived from said embryonic stem cells through gametogenesis to obtain a transformed gamete; and

obtaining progeny from the transformed gamete, thereby producing a transgenic non-human animal having a recombinant allele therein.

35. (Thrice Amended) A method for the production of recombinant alleles in a rodent, said method comprising:

introducing a nucleic acid fragment flanked by at least two recombination target sites into **[~~rodent~~]** embryonic stem cells **of [~~according to~~]** claim 26**, wherein said cells are rodent cells**,

passaging the genome derived from said embryonic stem cells through gametogenesis without causing recombination of the recombination target sites,

producing offspring resulting from crossing the genome of a gamete produced by the gametogenesis with the genome of a wild type rodent,

whereby the nucleic acid fragment is inserted into the genome of the offspring **and produces [~~so as to produce~~]** the recombinant allele therein.

40. (Thrice Amended) A method for the production of recombinant alleles, said method comprising:

introducing at least one nucleic acid construct into the genome of mammalian embryonic stem cells**,**

wherein said at least one nucleic acid construct comprises a germ**[-]**line- specific promoter operatively associated with a recombinase coding sequence, a nucleic acid fragment flanked by a **first [~~second~~]** pair of recombination target sites and a selectable marker flanked by a **second [~~first~~]** pair of recombination target sites,

passaging the genome derived from embryonic stem cells selected for expression of the marker through gametogenesis to obtain a transformed gamete; and

crossing the genome of the transformed gamete with the genome of a wild type animal, thereby obtaining first generation progeny wherein the marker is excised in the germ**[-]**line.

41. (Amended) A method according to claim 40 wherein said first pair of recombination target sites is recognized by a recombinase which is expressed under the control of a germ[-]line-specific promoter and said second pair of recombination target sites is recognized by a recombinase which is expressed under the control of an inducible promoter or a tissue specific promoter.

43. (Thrice Amended) A method for the conditional assembly of functional gene(s) for expression in eukaryotic cells by recombination of individual inactive gene segments from one or more gene(s) of interest,

wherein each of said segments contains at least one recombinase recombination target site, and wherein at least one of said segments contains at least two recombinase recombination target sites,

said method comprising:

introducing said individual inactive gene segments into a mammalian embryonic stem cell **of** **[~~according to~~]** claim 12 ,**wherein recombinase is expressed,** thereby **producing** **[~~providing~~]** a DNA which encodes a functional gene of interest, the expression product of which is biologically active, upon passage of the genome derived from said embryonic stem cells through gametogenesis.

44. (Thrice Amended) A method for the generation of recombinant non-human animal, said method comprising:

combining a nucleic acid construct comprising a germ[-]line-specific promoter operatively associated with a recombinase coding sequence with host pluripotential ES cells derived from early preimplantation embryos,

introducing these embryos into a host female, and

allowing the derived embryos to come to term such that a recombinant non-human animal is thereby produced by operation of the recombinase upon passage of the genome derived from the embryonic stem cell through gametogenesis.

49. (Amended) The **cells** **[~~method~~]** according to claim 12 wherein the **non-human mammalian** embryonic stem cell is a rodent cell.

50. (Amended) The **cells** **[~~method~~]** according to claim 49 wherein the rodent is a mouse.

51. (Amended) The **cells** **[~~method~~]** according to claim 12 wherein the **non-human mammalian embryonic** stem cell is a livestock stem cell.